New Mexico Nutrients: Translator Development Approach and Proof of Concept

Prepared for:

U.S. Environmental Protection Agency Region 6 1445 Ross Avenue, Suite 1200 Dallas, Texas 75202-2733

and

New Mexico Environment Department PO Box 26110 - 1190 St. Francis Drive N4050 Santa Fe, New Mexico 87505

Prepared by:

Tetra Tech, Inc. 73 Main Street, Suite #38 Montpelier, VT 05602

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Abstract

The purpose of this document is to present an analysis plan for developing numeric nutrient thresholds in streams in New Mexico. The plan primarily addresses nitrogen and phosphorus concentrations as they relate to relatively undisturbed site conditions, natural variations across site types, and potential thresholds that can be associated with protection of designated uses, primarily aquatic life uses. Data are being accumulated for a full scale analysis that will follow the plan. The scope of those data and preliminary analyses are presented in this report to demonstrate the analytical concepts. This report describes nutrient conditions in New Mexico streams, presents preliminary analyses of nutrient-biotic relationships, and suggests ranges of potential nutrient thresholds, but stops short of recommending thresholds.

The plan for nutrient criteria development includes 1) adoption of a conceptual model, 2) data organization and exploration, 3) data analysis to derive criteria, and 4) review, evaluation, and documentation of analyses. Existing conceptual models of nutrient effects in streams were reviewed and will provide the context of the relationships to be explored in the data. The data were assembled from three sources: the New Mexico Environment Department (NMED) monitoring programs, the U.S. EPA National Rivers and Streams Assessment (NRSA), and the U.S. EPA Wadeable Streams Assessment (WSA).

The proposed and partially tested analyses include descriptions of nutrient conditions in relatively undisturbed reference streams and relationships between nutrients and responsive indicators (dissolved oxygen, chlorophyll a, the periphyton assemblage, and the benthic macroinvertebrate assemblage). While the reference distribution analysis is fairly simple (depending upon adequate site classification), the stressor-response analyses are varied. Responses to nutrients were and will be analyzed using regression interpolation, correlation analysis, change-point analysis, propensity scores, species sensitivity distributions, and visualization tools for graphing distributions and relationships.

In the proof-of-concept section of the report, it was demonstrated that the preliminary analyses were not always conclusive and corroborating. Therefore, some refinements are necessary for implementing the full-scale analysis, including more detailed data organization for GIS variables and some indicators (especially dissolved oxygen and periphyton), scrutiny of the reference sites identified, improved site classification analysis, and execution and thorough interpretation of results for the multiple proposed stressor-response relationships and analyses. The final recommendations for nutrient criteria will be presented in a weight-of-evidence context, with ranges of potential criteria within site classes, confidence in each analysis, and interpretation of the expected protectiveness for selected criteria.

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1 Introduction

1.1 Background and Purpose

For nutrient concentrations in streams, the State of New Mexico currently has a narrative nutrient criterion and numeric criteria only for a few rivers. The narrative criterion in the *State of New Mexico Standards for Interstate and Intrastate Surface Waters* (Subsection E of 20.6.4.13 NMAC) is as follows:

Plant nutrients from other than natural causes shall not be present in concentrations which will produce undesirable aquatic life or result in a dominance of nuisance species in surface waters of the state.

The narrative nutrient criteria can be difficult to implement because the relationships between nutrient levels and impairment of designated uses are not defined, and distinguishing nutrients from "other than natural causes" is difficult (NMED 2006). Therefore, the Surface Water Quality Bureau (SWQB) has developed nutrient assessment protocols for streams and is planning to refine the protocols and nutrient threshold values with regional data and verified classification systems.

The purpose of this document is to present an analysis plan for developing numeric nutrient thresholds in streams in New Mexico. The plan will primarily address nitrogen and phosphorus concentrations as they relate to relatively undisturbed site conditions, natural variations across site types, and potential thresholds that can be associated with protection of designated uses, primarily aquatic life uses. Data are being accumulated for a full scale analysis that will follow the plan. The scope of those data and preliminary analyses are presented in this report as proof of the analytical concepts. This report describes nutrient conditions in New Mexico streams, presents preliminary analyses of nutrient-biotic relationships, and suggests ranges of potential nutrient thresholds, but stops short of recommending thresholds. The results presented here are in support of the State's effort and should only be used as components of a broader and continuing analysis.

The development approach for nutrient translators will use a stepwise process for using reference conditions and stressor-response relationships to derive nutrient criteria (USEPA 2009). Although the approach cannot be fully implemented yet, the feasibility of analytical elements of the approach are demonstrated to verify that the concepts have the potential of being used. The proof-of-concept addresses the core principles using complete datasets, but the analyses may not address all datasets. These preliminary analyses are intended to highlight potential problems and opportunities that may influence the analysis plan.

1.2 Nutrients in New Mexico Streams

Nutrients occur in streams naturally and can be greatly increased due to human activity. In this study we focus on nitrogen and phosphorus because these nutrients (or other non-nutrient

factors) typically limit or enhance primary production and are readily measured. Other nutrients are usually only required in trace amounts for plant growth and are rarely limiting to production. Therefore, increases in nutrients other than nitrogen and phosphorus may be evident with increased human disturbance, but they are not suspected of causing changes in the primary and secondary producers.

Human activities can cause increases in nutrient concentrations in streams through a variety of pathways. These include, but are not limited to, fertilizer application, soil and vegetative disturbance, partial treatment of wastewater, and animal production. Increases in major nutrients are often associated with increases in other pollutants and stressors. Nutrients may be associated with turbidity and Total Suspended Solids (TSS). Suspended sediments are associated with the benthic macroinvertebrate assemblage (Jessup et al. *in preparation*). The interaction of multiple stressors can cause amplified or buffered effects on responding organisms. This phenomenon was partially explored in this analysis, though the emphasis remains on the interaction between major nutrients and biotic responses.

The forms of nitrogen and phosphorus vary depending on the conditions in their environment. Some forms, such as ammonium ions (NH₄) or nitrate ions (NO₃) and orthophosphate (PO₄), are more accessible for uptake by plants. However, the best indication of potential nutrient availability is the sum of all forms, or total nitrogen (TN) and total phosphorus (TP). Typical nutrient measures from streams samples include nitrate (NO3), nitrite (NO2), combined NO3+NO2, ammonium (NH4), total Kjeldahl N (TKN), TN, orthophosphate, and TP. The most common measures are NO3+NO2, TKN, TN, and TP. TN can be calculated from TKN + NO3+NO2.

1.3 Aquatic Life

Protection of aquatic life uses is the impetus for establishing nutrient criteria. The pathways by which nutrient concentrations affect aquatic life conditions are complex, as suggested in conceptual models (e.g., EPA 2010, EPA 2012) and literature supporting linkages along the pathways. The basic relationships are described here and form the basis of our argument for using available response measures in the proposed analyses.

Nutrient impaired waters can cause problems that range from annoyances to serious health concerns (Dodds and Welch 2000). In streams, gross primary production is effected by to nutrient concentration, especially phosphorus (Mulholland 2001). The primary producers include periphyton and aquatic macrophytes. Periphyton (including diatoms) are ubiquitous in streams and can be sampled consistently. They are therefore potential indicators of nutrient conditions. Chlorophyll *a* on substrates or in the water column is an estimate of algal biomass. Periphyton species are responsive to stressors other than nutrients, especially in the West (Stevenson et al. 2008), but these confounding factors may be recognized and perhaps even factored out of descriptive stressor-response relationships.

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Benthic macroinvertebrates interact directly with periphyton, and therefore, indirectly with nutrients. There may be some direct nutrient – macroinvertebrate response pathways, but these are not well defined. The indirect effects are through pathways of respiration (oxygen supply) and food availability. Periphyton provide oxygen when photosynthesizing, but can deplete oxygen as well, when microbes respire in the decay of excessive periphyton, caused by excessive nutrients. Production and respiration in streams are related to thecan be assessed through examination of the diel DO range, or deficit, in streams (Mulholland 2005). Therefore, measures of oxygen are most useful when taken frequently over a series of days. The pattern of oxygen production and depletion can then be associated with nutrients more comprehensively than single point measures.

Macroinvertebrates can graze and inhabit periphyton communities. Some grazers may prefer certain types of periphyton. Excessive periphyton can degrade macroinvertebrate habitat for those organisms that require substrates with sparse algal growth. Therefore, the indirect effects of nutrients on benthic macroinvertebrates, through periphyton, can cause varied responses in the macroinvertebrate community. These interactions can occur in both directions – with macroinvertebrates effecting periphyton through selectively grazing, sometimes to a degree that affects nutrient uptake from the water column. Benthic macroinvertebrates are responsive to many stressors other than nutrients, and the possible confounding effects should be factored out, when they are recognizable. In a study of stream conditions in the Ozark Highlands Ecoregion in Arkansas, biotic indices for three biotic assemblages were negatively correlated to nutrient concentrations (Justus et al. 2010). The algal index had a higher correlation (rho = 0.89) than did the macroinvertebrate and fish indices (rho = 0.63 and 0.58, respectively). This suggests that the more direct effects with few indirect factors were reflected in the stronger correlations.

1.4 Natural Variability

Natural nutrient concentrations can be inferred from conditions observed in streams with minimal human activity at the site and in the catchment. These are referred to as the reference conditions (Barbour et al. 1999, Stoddard et al. 2006). Reference nutrient conditions are subject to unavoidable human activities (such as atmospheric deposition), availability of suitable reference sites, and adequate recognition of natural variability. Reference sites can be identified using objective criteria for the stressors and stressor sources measured at the site or sensed remotely and analyzed using GIS. While quantitative criteria are defensible and repeatable, subjective input from knowledgeable experts is necessary to confirm final reference site selection.

Nutrient concentrations in New Mexico streams are expected to have relatively homogenous concentrations within homogenous landscape types. The level III and IV ecoregions of New Mexico (Griffith et al. 2006) may be the best indicators of landscape types that affect reference nutrient conditions, as indicated by previous analyses that distinguished five site classes (NMED 2011). In addition, NMED recognizes cold-water, warm-water, and transitional designated uses, which are considered as another layer of site classification. Analyses were distinguished by ecoregional groups and designated uses when sufficient data existed.

1.5 Existing Nutrient Thresholds in New Mexico

In 2002, the NMED Surface Water Quality Bureau (SWQB) developed a nutrient assessment protocol (NMED 2006). It addressed both causal and response variables but did not result in quantifiable measures. In 2004, SWQB with the assistance of EPA and the U.S. Geological Survey (USGS) refined the protocol. Analysis of existing data and a literature review was conducted to develop impairment threshold values for each of the variables used in the assessment protocol. TN and TP threshold values were calculated (based on percentile selections) for Level III Ecoregions in New Mexico. The current protocols for assessing impairments in streams (NMED 2011) include screening level thresholds for TN and TP (Table 1).

Table 1. NMED's nutrient thresholds for wadeable, perennial streams (mg/L). Reproduced from NMED (2011).

| | | 21- | 20 | /22- | | 23- | 24/79- | | 25/26- | |
|--------|------|---------------------|-------|-------|------|---------|------------|------|---------|------|
| | Sout | thern Rockies | AZ | /NM | AZ | Z/NM | Chihuahuan | So | uthwest | tern |
| | | | Plate | eau** | Mou | ıntains | Desert** | T | ablelan | .ds |
| ALU* → | CW | T/WW (volcanic ***) | CW | T/WW | CW | T/WW | T/WW | CW | T | WW |
| TN | 0.25 | 0.25 | 0.28 | 0.48 | 0.25 | 0.29 | 0.53 | 0.25 | 0.38 | 0.45 |
| TP | 0.02 | 0.02 (0.05) | 0.04 | 0.09 | 0.02 | 0.05 | 0.04 | 0.02 | 0.03 | 0.03 |

 \underline{NOTES} : * ALU = designated aquatic life use of the assessment unit

CW - streams with only coldwater uses (high quality coldwater or coldwater)

T - transitional streams with marginal coldwater, coolwater, or both cold and warmwater uses

WW – streams with only warmwater uses (warmwater or marginal warmwater)

- ** Because of the limited area and number of sites in the Madrean Archipelago (79) and Colorado Plateau (20) ecoregions, these data where grouped with the most similar ecoregions; the Madrean Archipelago with the Chihahuan Desert and the Colorado Plateau with the Arizona/New Mexico Plateau. The Western High Plains (25) had no stream data as the only surface waters are playas, therefore this protocol does not apply to this ecoregion.
- *** The volcanic threshold is applicable to Level IV ecoregions 21g and 21h as well as 21j in the Jemez Mountains

The relationship between nuisance algal growth and nutrient enrichment in stream systems has been well documented in the literature (Van Nieuwenhuyse and Jones 1996; Dodds et al. 1997; Chetelat et al. 1999, Suplee et al. 2008, Stevenson et al. 2006). The NMED assessment protocols (2011) currently include procedures for assessing algal growth using visual assessments and measures of dissolved oxygen, pH, and chlorophyll a. For dissolved oxygen and pH, thresholds are established for a screening level (Level 1) and a more detailed Level 2 assessment. For the screening level, NMED assumes that high rates of primary production can cause D.O. supersaturation and high pH during the day. Impairment is suspected if D.O. saturation readings above 120% and pH values above the appropriate aquatic life criterion (i.e., pH > 8.8 for high quality cold and coldwater uses or pH > 9.0 for marginal cold, cool, warm, and marginal warmwater uses). In the Level 2 assessment, D.O. and pH data are collected using multi-parameter, continuous recording devices (sondes) to observe diel fluctuations, as opposed to the "snapshot" that one-time data provide. Because algal biomass above nuisance levels often produces large

diel fluctuations in dissolved oxygen (D.O.) and pH, D.O. concentration, percent local D.O. saturation, and pH are used as indicators of nuisance levels of algal biomass. In a Level II nutrient assessment, fluctuations in D.O. data that exceed 3 mg/L can be indicative of excessive algal activity. For chlorophyll *a*, Level 2 assessment includes collection of an algal sample, analysis of chlorophyll a concentration and comparison of the concentration to thresholds (Table 2).

Table 2. Chlorophyll *a* Level III Ecoregional Threshold Values in μg/cm². Reproduced from NMED (2011)

| (2011). | | | | |
|-------------|-------------|------------|-------------|------------|
| 21-Southern | 20/22-AZ/NM | 23-AZ/NM | 24/79- | 25/26-SW |
| Rockies | Plateau | Mountains | Chihuahuan | Tablelands |
| | | | Desert | |
| 3.9 - 5.5 | 7.4 - 7.8 | 5.8 – 11.0 | 16.5 – 17.5 | 8.2 - 14.0 |

Note: Since the number of samples used to calculate the thresholds is relatively small for each ecoregion, the 90th to 99th percentile range is used for threshold values.

1.6 General Approach

There are three general types of empirical analyses that can be used to derive numeric criteria: (1) the reference condition approach, (2) mechanistic modeling, and (3) stressor-response analysis (US EPA 2000). An additional component of these analyses is scientific literature review for support or suggestion of criteria. When multiple analyses are used as corroborating lines of evidence, resulting criteria are most defensible. For deriving nutrient criteria in New Mexico streams, we propose using three approaches: all of those mentioned except mechanistic modeling, which would require more intensive research than is currently planned.

The reference condition approach defines expectations for nutrient concentrations based on observations of conditions in reference waterbodies (USEPA 1998, 2000, Barbour et al. 1999, Stoddard et al. 2006). Reference waterbodies represent least disturbed and/or minimally disturbed conditions within a region (Stoddard et al. 2006) and that ideally support designated uses (US EPA 2000a). Criteria for a particular variable (e.g., total phosphorus or total nitrogen) are derived by first describing measurement distributions from reference waterbodies and then selecting a representative value to define expectations for reference and for all other waterbodies. The reference condition approach requires confident definition and identification of reference waterbodies, accounting for natural variability within site types, and availability of sufficient data from these reference waterbodies to characterize the distributions of nutrient variables.

Stressor-response approaches refer to analytical techniques that derive candidate endpoints by exploring and identifying thresholds in the relationships between response variables and nutrient concentrations. Typical response variables in the context of nutrient endpoint development include biomass and assemblage metrics (e.g., percent nutrient sensitive diatoms) and aquatic life use indicators or biocriteria indicators (e.g., trophic state indices, algal multimetric indices, or invertebrate multimetric indices). The value of these indicators is their direct linkage to aquatic life use designations. Therefore, they provide a way to connect nutrient concentrations directly to aquatic life use protection.

In EPA's draft guidance on Empirical Approaches for Nutrient Criteria Derivation (USEPA 2009), several methods for evaluating stressor-response relationships were presented. The approaches implemented in this analysis were adopted to take advantage of available data and to produce robust results using a combination of well-established and exploratory analytical techniques. The focus of the analysis was on the major nutrients, nitrogen and phosphorus, as they relate to the available response measures, periphyton, chlorophyll a, dissolved oxygen, and benthic macroinvertebrates.

The analytical techniques recommended for relating stressors and responses included regression interpolation, correlation analysis, change-point analysis, species sensitivity distributions, and propensity scores. We describe these techniques and the relative importance of each, including the advantages and limitations that lead to differential weighting of potential nutrient criteria analyses.

1.7 Data Description

Data were compiled from three main sources (Table 3). The NMED data were most numerous and were collected for various projects over time. The nutrient records were most numerous and complete, while other types were relatively sparse and only used in this analysis when associated with nutrient information. Data compilation and analysis were and will be conducted following the Quality Assurance Project Plan (QAPP) that was developed specifically for this project (Tetra Tech 2012).

Table 3. Data summary by source.

| NMED: | 883 valid sites in NM with water chemistry |
|--|---|
| | Multiple samples per site (approximately 7352 samples) |
| | Years 1990 - 2012 |
| | Chemistry, site & habitat characteristics |
| | Benthic macroinvertebrate samples in 141 sites |
| | Periphyton in 212 sites |
| | Chlorophyll <i>a</i> in 146 sites |
| | Dissolved oxygen diel data in approximately 200 sites |
| NRSA: 88 sites, each with a single visit | |
| | Years 2008 - 2009 |
| | 44 sites in NM, others within 50-150 miles of NM |
| | Chemistry, Chlorophyll a, periphyton, site & habitat characteristics |
| WSA: 56 sites, each with a single visit | |
| | Years 2000 - 2004 |
| | 10 sites in NM, others within 50-150 miles of NM |
| | Chemistry, benthic macroinvertebrates, site & habitat characteristics |

Nutrient Data

The nutrient database included more than 7,000 records of nutrient concentrations in 883 NMED stream sites. Samples were collected through the years from 1990 to 2012. Samples were much

less common in winter months (December, January, and February). The nutrients recorded were related to nitrogen (ammonia, nitrate + nitrite, and TKN), phosphorus (orthophosphate and total phosphorus) and ancillary analytes (pH, specific conductance, temperature, turbidity, and dissolved oxygen). Total nitrogen was calculated as nitrate + nitrite + TKN. Ammonia was commonly measured in the field, but was not analyzed in the laboratory. Measures of total phosphorus were roughly 24x more common than measures of orthophosphate, and therefore orthophosphate was not analyzed. For these analyses, "non-detect" data points were given a value equaling 50% of the most common detection limit.

Nutrient data from the NRSA included total nitrogen and total phosphorus as well as nitrate, nitrite, and ammonium. These data plus information on the ancillary analytes pH, specific conductance, temperature, turbidity, and dissolved oxygen were complete for all 88 sites, half of which were in New Mexico. WSA + EMAP nutrient data included total nitrogen and total phosphorus as well as nitrate and ammonium. Water chemistry, including ancillary analytes, was complete for 56 sites, 10 of which were in New Mexico.

Macroinvertebrate Data

The NMED benthic data are stored in a relational database, the Ecological Data and Application System (EDAS). The database is used to calculate macroinvertebrate metrics and indicators of water quality. EDAS contains raw macroinvertebrate taxa lists, Operational Taxonomic Units (OTUs), calculated indicators (metrics and multimetric indices) for the majority of sites. About 141 NMED sites were found with macroinvertebrate and chemistry samples collected within 30 days of each other. These macroinvertebrate samples were collected using six different methods, which will need to be assessed for comparability. WSA + EMAP benthic data (collected with one consistent method [Peck et al. 2006]) were summarized as metrics in spreadsheet format. In the WSA + EMAP dataset, 56 benthic samples matched the chemistry samples. NRSA benthic data were not yet available.

NMED benthic samples were the basis for calculation of the New Mexico Macroinvertebrate Stream Condition Index (NMMSCI) (Jacobi et al. 2006) and other metrics that were components of the NMMSCI or otherwise believed to be responsive to sediment stresses in New Mexico streams. Because the NMMSCI was originally calibrated with midges (Diptera: Chirnomidae) at the family level, the calculations for this analysis also included family-level midge data. All other metrics counted midges and all other taxonomic groups at the level identified by the laboratory taxonomist (mostly genus-level). The NMED is using the NMMSCI in mountain streams for assessment purposes, but does not use it in other site types (personal communication James Hogan, NMED). If benthic macroinvertebrate data existed from several sampling events, the metrics from the sampling event that coincided with the sediment sample were used. Where both targeted riffle and reach-wide sediment samples were collected, they were only compared to benthic samples collected from the same habitat type.

One benthic macroinvertebrate sample was compared to average site chemistry from samples collected within 30 days of the benthic sample. If multiple benthic methods were used on a single date, a preferred method was selected, with preferences as follows: Kick > Targeted Riffle > Reachwide > other. The preferences were established to maximize sample size, increasing the

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Comment [pc5]: Seems odd that only 10 of the 56 sites was in NM.

likelihood of analyzing the complete stressor gradient. We analyzed metrics and indicators with proven responses to stress.

Macroinvertebrate data will be analyzed by ecoregion, designated use, and sampling method. Opportunities to aggregate samples collected by different methods will be explored and samples from multiple methods will be pooled when the results of each method overlap in stressor-response bi-plots. Separate analyses will be conducted for methods that cannot be aggregated because of non-overlapping data points in the bi-plots.

Periphyton Data

Periphyton data in and around New Mexico were collected by NMED and the NRSA. Through the NMED, roughly 212 diatom samples were collected from 2002 to 2008 mostly in the fall sampling season (August - November). Soft algae samples were collected from 133 sites. Periphyton data from 69 NRSA sites in and around New Mexico were added to a single periphyton database. The NRSA periphyton samples include both diatoms and soft algae. Potential bias that may be introduced by different sampling protocols will be investigated.

In the NRSA dataset, 22 metrics have been calculated on the periphyton assemblage. For preliminary analyses, these metrics can be analyzed. For the NMED diatom data, metrics need to be calculated. In similar analyses, Tetra Tech has calculated approximately 100 diatom metrics in a relational database. These included metrics described by Porter et al. (2008), by Stevenson et al. (2008), Kelly and Whitten pollution tolerance index (1995), Van dam metrics (1994), and periphyton indices developed by Potapova and Charles (2006). These metrics can be calculated and tested in the New Mexico datasets.

Chlorophyll a

Of the NMED wadeable stream sites with nutrient data, 146 also had chlorophyll *a* data (including 35 with benthic macroinvertebrate data as well). These samples occurred between 1994 and 2011 in the months of August to November.

Diel Dissolved Oxygen

Diel dissolved oxygen data were collected in approximately 200 stream sites throughout New Mexico. These data were collected along with pH using multi-parameter, continuous recording sondes with recording periods of at least 48 hours. The data are in multiple spreadsheets. They need to be checked for errors and combined in a single database so that metrics can be calculated efficiently. Errors that typically occur with sonde data relates to records before and after the sonde is placed in the water or in association with drifting calibration.

Site characteristics

Site characteristics are in four categories: observed or measured data versus remotely sensed data and human disturbance variables versus natural condition variables. The observed or measured data includes physical habitat measures of instream, channel, and riparian conditions. These data were recorded during site visits and include physical habitat assessments, channel dimensions, slope, canopy cover, riparian vegetation, riparian integrity, substrate characterizations, and more. These data are ancillary to the nutrient data and can be associated for analysis of covariates, buffers, and synthesizing effects. Each data source included somewhat different variables for the observed and measured site characteristics.

The remotely sensed data can be derived from Geographic Information System (GIS) analysis. These data include information on the setting of the sampling site and surrounding areas, such as ecoregion, land use types and intensity, roads and road crossings, population density, watershed area, and more. GIS analysis can be time consuming and therefore it may not be feasible to include all sampling sites in the analysis. The GIS analysis should be carried out on a subset of sites with complete datasets or of particular interest. In this preliminary analysis, we identified two types of sites with high priority for GIS analysis, as follows.

- Sites with comparable response data. Sites that have benthic macroinvertebrate, chlorophyll a, periphyton (diatom), or diel dissolved oxygen data can be used to detect biological responses to nutrient conditions. At present, the chlorophyll a and benthic macroinvertebrate data are available. The diel dissolved oxygen and periphyton data are not.
- 2. Reference (least disturbed) sites. These will be used to characterize background nutrient conditions in undisturbed sites. GIS is sometimes used to identify or screen disturbance intensity at sites, so the potential reference sites will be identified in other ways first and confirmed after GIS analysis. Priority will be given to sites with habitat and flow data that would help in site classification.

A total of 393 sites were recommended as high priority for GIS analysis based on the guidelines above (**Appendix A**). The analysis was conducted by EPA Region 6. The watershed characteristics for the preliminary analysis were not taken in the upstream catchment of the site because time constraints did not allow delineation of all the watersheds. Instead, a 1.6 km (1 mile) radius buffer around the site reach was delineated and land use and other areal characteristics were estimated in that oblong area.

1.8 Criteria Development Process

The entire criteria development process begins with problem recognition and can ultimately end with adoption of nutrient standards into state law or regulation. The focus of the steps we outline are on the technical aspects of criteria development, with less emphasis on the early conceptual and organizational steps or the later programmatic implementation steps. Therefore, the steps we consider include 1) development of a conceptual model, 2) data organization and exploration, 3) data analysis to derive criteria, and 4) review, evaluation, and documentation of analyses (U.S. EPA 2010). This report is primarily concerned with step 2, data organization and exploration, though the contexts of earlier and later steps are important to guide the data exploration.

Conceptual models are developed to represent known relationships between changes in N and P concentrations, biological effects, and attainment of designated uses. Conceptual models are well established and are not reconstructed for this presentation. Instead, readers are referred to the conceptual model published by EPA (2010) as a standard that is applicable in New Mexico streams (Figure 1). These conceptual models show intricate pathways of effects. Our analyses sometimes rely on the validity of each linkage in the pathway, as when indirect effects are directly related (e.g., relating nutrient concentrations to macroinvertebrate responses). The

conceptual models illustrate interactions that may be occurring when we cannot account for them due to analytical constraints (e.g., missing data elements). They not only provide a means of communicating the current state of knowledge regarding the effects of N and P in aquatic systems, but also provide an important tool for guiding subsequent analyses. Literature review and summary are important in developing and substantiating the relationships illustrated in the conceptual model.

In the second step of the process, variables are selected for analysis, data are assembled, and characteristics of these data explored. The current project is focused on this step, which is in preparation for more intensive analyses. Data regarding nutrients, water chemistry, physical habitat conditions, site characteristics, and response variables were compiled from multiple sources. Data exploration consisted of preliminary analysis using techniques intended for final analysis once the datasets are fully assembled. The preliminary analyses and data visualization tools will be used to select variables and appropriately quantify the stressor (i.e., excess nutrients) and the response. This will guide the focus of later analysis by highlighting potential problems, uncertainties, and meaningful relationships.

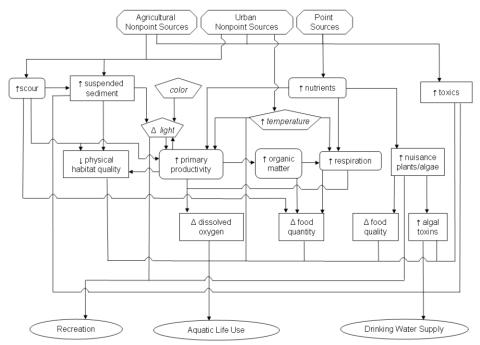


Figure 1. Conceptual diagram linking sources of human disturbance with designated uses through pathways that include nutrients (from U.S. EPA 2010).

The third step includes data analysis to estimate the critical stressor-response relationships depicted in conceptual models and to identify thresholds that may be used to derive water quality criteria. A three-stage approach to analysis is recommended, in which waterbodies are first classified, stressor-response relationships are estimated within each class, and criteria are derived from the estimated relationships (U.S. EPA 2010). This report outlines the analysis plan to be implemented, including descriptions of the recommended analyses and interpretation of preliminary results from their application.

Fourth, analyses are reviewed, evaluated and documented. This may include validation of predictive performance for a stressor-response model and selecting a model that best represents the data. This step allows for transparent justification of criteria as they are considered for adoption into state standards.

2 Datasets:

Data were collected from three sources: NMED, the National Rivers and Streams Assessment (NRSA) and the Wadeable Streams Assessment and Environmental Monitoring and Assessment program (WSA & EMAP) (Table 3). The NRSA and WSA & EMAP data were collected by the U.S EPA. All data were compiled in a single relational database (Microsoft Access), though data from each source were maintained in separate database tables.

The NMED monitored four primary water quality variables (TN, TP, chlorophyll a, and turbidity) in New Mexico streams plus a number of secondary variables including DO concentration, DO percent saturation, pH, and AFDM (NMED 2006). In addition, SWQB conducted biomonitoring of benthic macroinvertebrate, periphyton, phytoplankton, and fish community composition at select sites. Biomonitoring samples were collected in accordance with the methods documented in the EPA Rapid Bioassessment Protocol For Use in Wadeable Streams: Periphyton, Benthic Macroinvertebrates, and Fish (RBP) (Barbour et al. 1999), Stream Periphyton Monitoring Manual (Biggs and Kilroy 2000), and/or the NMED Standard Operating Procedures (SOP) (SWQB/NMED 2005). The NMED data used in these analyses included four databases: nutrients, periphyton, macroinvertebrates, and dissolved oxygen. Each database included data from sites throughout the state that were identified with a Station ID and latitude/longitude coordinates. The Station ID was primarily used to link information among databases. Dissolved oxygen data were not organized in time for the preliminary analyses.

Data were collected as part of the NMED nutrient criteria development projects as well as for regular water quality surveys. Some of these data were targeted to focus on reference reaches for stream classification and for identifying threshold values for nutrients, algal biomass, and secondary variables (NMED 2006). The streams were selected to span at least five ecoregions throughout the state. Monitoring focused primarily on a critical low flow index period from August to November. At some sites, the monitoring plan allowed examination of seasonal and annual variability and trends.

NMED collected and processed samples in accordance with methods documented in an EPA approved Quality Assurance Project Plan (QAPP) and associated Standard Operating Procedures (SOP). The QA/QC procedures in the QAPP included collection and analysis of replicates for 10% of water samples, adherence to calibration methods, and taxonomic verification of a subset of periphyton and benthic macroinvertebrate samples. Also included was a thorough QA review of all site and analytical data, including flagging of all parameters that were outside of the control limits.

NRSA and WSA & EMAP data were transferred from the U.S. EPA. They included information regarding water chemistry, physical habitat, and biological assemblages. For the NRSA, information on benthic macroinvertebrates, periphyton, and chlorophyll a were available. For the WSA & EMAP data, benthic macroinvertebrates were the only biological data available. Data for NRSA were collected in accordance with the Field Operations Manual (U.S. EPA 2007). For WSA & EMAP, data were collected using methods similar to those used in the NRSA, following procedures outlined by the U.S. EPA (2004) and Peck and others (2006).

A QAPP was developed for this current project (Tetra Tech 2012), primarily to address procedures for working with secondary data. Procedures for data compilation and analysis were followed to

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minimize errors in transferring data from original sources into analytical databases. Although data were expected to be error-free in the original form, checks for outlier values were implemented and unexplainable outlier values were removed from analysis.

3 Analytical Methods

The analytical methods to be performed are in two major categories: indicator value distributions emphasizing reference conditions and the stressor-response approach. The reference condition approach includes identification of minimally disturbed sites, classification of the sites, and description of the reference condition based on characteristics in those sites (USEPA 1998, 2000, Barbour et al. 1999, Stoddard et al. 2006). In this approach, we derive candidate criteria from distributions of nutrient concentrations in reference waterbodies, in this case, streams. Reference streams represent least disturbed and/or minimally disturbed conditions (Stoddard et al. 2006) and share similar characteristics to the waterbodies for which criteria are being derived. Criteria are derived by selecting percentile values from the distributions of nutrient concentrations in reference sites or in all sites. USEPA's nutrient criteria guidance recommends the use of percentiles derived from the reference waterbody distributions, since these waterbodies represent an example of the biological integrity expected for a region (USEPA 2000). Site classification is inherent to the reference condition approach, in which variability due to natural conditions is identified in categories or on a continuous scale with a natural environmental variable.

The stressor-response approach involves estimating a relationship between nutrient concentrations and biological response measures related to designated use of a waterbody (e.g., a biological index or recreational use measure) either directly or indirectly, but ideally quantitatively. Then, nutrient concentrations protective of designated uses can be derived from the estimated relationship. This approach relates nutrient concentrations to response measures and thus to designated uses and is the focus of this document.

3.1 Reference Site Identification

Reference stream sites have been identified in and around New Mexico for multiple purposes, including nutrient citeria development (NMED 2006), biological index development (Jacobi et al. 2006, Paul 2008), sediment threshold estimation (Jessup et al. 2010), and the national stream surveys. The designations established for each purpose were adopted to create a list of potential reference sites for this project. These sites will be further analysed using GIS to confirm the designation for the nutrient analysis. While the reference designations established for NMED projects did not use nutrient measures as criteria for ranking disturbance levels, those for the national surveys did (Kaufmann et al. 2012). If used as provided (which we do not recommend, but have done in the preliminary analyses), the reference statistics would exclude sites that have high nutrient concentrations but lack other indications of disturbance. Instead, the reference designations should be recalculated to reflect disturbances that are independent of nutrients and therefore sites with high nutrient concentrations may be included in the reference dataset.

Reference sites will be identified through corroboration of designations used in previous studies and reference site criteria developed using GIS variables. In addition, the NRSA and WSA + EMAP criteria will be reapplied after removing criteria related to nutrient concentrations. Sites with contradictory indications of reference status from the multiple techniques will be relegated

to the non-reference category. Because the NMED staff are familiar with site conditions that may not be reflected in the data, they will have veto power over the reference designations indicated through empirical analyses.

If reference sites are notably lacking in some parts of New Mexico, we will weigh the consequences of excluding those areas from analysis because of ubiquitous disturbance or including sites with disturbance as the reference dataset as the "best available" reference. The decision to exclude whole areas will depend on our confidence that the expectation for streams in those areas should be the same as remote reference streams. If the streams in areas with ubiquitous intensive land use are somehow unique, the best available reference designation should be recognized so that nutrient expectations based on them will reflect disturbance levels.

In some analyses, identification of sites that are stressed by intensive human disturbance in the catchment, degraded habitat conditions, or water quality issues other than nutrients. If these sites can be identified, they may be specifically included to interpret nutrient sources or specifically excluded to control for extraneous factors when interpreting nutrient-response relationships. Multiple stressors may be common in sites with high concentrations of nutrients. Finding stressor-response relationships that are attributable to nutrients alone will be depend on controlling for the other stressors.

3.2 Site Classification

Site classification is the process by which natural gradients among sites are examined to identify sites with similar nutrient conditions in the absence of human disturbance. The purpose of classification is to minimize within-class natural variability of indicators so that anthropogenic disturbance can be recognized with less background noise (Barbour et al. 1999, Hughes et al. 1995). Potential site classification variables, nutrient indicators, and biological variables will be analyzed simultaneously to identify patterns of covariance that could suggest how nutrient conditions could be classified according to environmental and biological characteristics.

NMED initially classified stream sites for application of nutrient thresholds using groupings of similar Level III ecoregions and by designated use (dividing waters within each ecoregion into warm, transitional [i.e., segments with both cold and warm water designated uses], and coldwater aquatic life uses) (Table 1, NMED 2011). These site classifications will be examined to determine if they are still valid with the combined datasets. The classification scheme developed for sediment assessments (Jessup et al. 2010) will also be tested. Additional classification categories will also be examined as potential improvements on the existing scheme. The other classification variables will include Level IV ecoregions (Griffith 2006), geology, stream order, elevation, width/depth ratio, entrenchment ratio, sinuosity, channel materials, and stream slope, among others.

For example, phosphorus and light interact to effect algal growth (Hill and Fanta 2008, Hill et al. 2009, Mulholland 2001). Under controlled conditions it takes very little P to maximize algal growth given high light. This fundamental relationship may be observed in our datasets, suggesting that natural stream shading may be a classification variable when considering stressor-response relationships. However, the relationship may not be observed if algal production is limited by other

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factors such as bottom substrate, turbidity, canopy cover, hydrology, or depth. The degree of refinement of the classification scheme may be limited by the number of samples, especially reference samples, available for analysis.

The first step in site classification is defining the data frame, or population of streams from which data will be analyzed and to which resulting criteria can be applied without extrapolation. The waterbodies of interest for this effort include perennial wadeable streams in New Mexico on-in close proximity and similar ecoregions of neighboring states. It does not include ephemeral or intermittent streams, springs, and direct WWTP effluent. Also excluded are large rivers, that cannot be monitored effectively with methods developed for wadeable streams and generally have drainage areas greater than 2,300 square miles (NMED 2011). The systems considered to be large rivers, and consequently exempt from this protocol, include:

- San Juan River from below Navajo Reservoir to the Navajo Nation boundary near Four Corners,
- 2. Animas River from the Colorado border to the San Juan River,
- 3. Rio Grande in New Mexico,
- 4. Pecos River from below Sumner Reservoir to the Texas border,
- 5. Rio Chama from below El Vado Reservoir to the Rio Grande,
- 6. Canadian River below the Cimarron River, and
- 7. Gila River below Mogollon.

The classification analysis should include several methods, including principal components analysis, correlation analysis, and examination of bi-plots and distributions.

Principal components analysis (PCA) can be used as a primary tool for selecting site classification variables. The PCA can be run in two configurations: reference sites alone and all sites. When only reference sites are used, natural, nutrient, and stressor variables can be included as determinants. When all sites are included, only natural variables should be included. The advantage of using all sites is that regions with fewer reference sites will be represented. When included as supplemental variables (not influencing the organization of principal components), the stressor, biological, and nutrient variability can be compared to the principal axes. We can examine nutrient-related axes that are correlated with biotic variables to gain insight into potential scaling or classification variables that would minimize biological variability and thus focus biological responses on disturbances. Variables should be transformed as needed to approximate normal distributions using logarithmic and Arcsine-Square Root transformations. Ecoregion designations are difficult to use in PCA because the values are categorical, not continuous.

Correlation analysis can be used to describe single factor relationships between nutrient and environmental variables in reference sites. The Spearman rank order correlation coefficient will be less sensitive to skewed distributions and can be calculated for a matrix of individual variables (sediment, natural, stressor, and benthic metrics). In contrast to the PCA, the correlation analysis should be limited to reference sites to emphasize the effects of natural site conditions instead of disturbance levels. Partial correlations can also be attempted if the variables are transformed to approximate normal distributions. Partial correlations emphasize relationships after factoring out covariants.

The relationships that are suggested by PCA and correlations should be examined in box plots and bi-plots. For example, the distribution of nutrient concentrations in reference sites of the existing site classes can be plotted and examined for precision within classes and differences among classes. Distributions that show high variability within a class may indicate a need for more refined classification. Classes with similar interquartile ranges show possibilities for combining classes. If the box plots show precise and distinct distributions then confirmation of classes is indicated. Bi-plots can be used to show patterns of relationships between variables and to highlight tertiary attributes of the relationships such as reference status, ecoregion, or other covariants.

It is likely that multiple factors effect response variables. Those that have been suggested include stream shading, flow, substrate, turbidity, and others. The degree to which these other factors mask or accentuate responses between nutrients and response measures can sometimes be recognized and factored out. However, extensive analysis to recognize possible factors can suggest data parsing that is unreasonable for the sample sizes we have available for analysis. If we reduce the nutrient-periphyton analysis to one type of sample with similar characteristics (e.g., NRSA sites with >75% canopy, cobble dominated substrates, in cold water Southern Rockies streams), we might have very few data points from which to find meaningful relationships. Adjustment of variables based on regression residuals of the extraneous factors can be useful if the regression is strong enough.

3.3 Nutrient Distribution Descriptions - The Reference Approach

The distributions of nutrient concentrations will serve as a baseline description of nutrient conditions throughout New Mexico. The distribution percentiles in different subsets of the data can be used to describe general nutrient conditions by nutrient species, ecoregion, or reference status of the sites. These standards (percentiles of distributions in site categories) have long been established (U.S. EPA 1998, U.S. EPA 2000, Barbour et al. 1999) and are now accepted as practical guidelines for describing reference expectations.

The 75th percentile of reference sites within site classes is a common value used for deriving potential criteria for nutrients. At this level, a lower value passes the criterion and indicates that the observation is similar to the lower 75% of nutrient concentrations. If confidence in reference sites and site classes is extremely high, less error in the reference values should be expected and a higher percentile of the distribution (such as the 90th) could be used as a guideline for establishing criteria. If confidence is low, as when the best available reference sites are selected for a region that has a generally high intensity of disturbance, then more error in the reference sites can be expected and a lower percentile (e.g., the 50th) can be recommended. An observation in the site class with low confidence in the reference conditions will only pass if it is similar to the best 50% of reference values.

We do not expect that the reference site selection process or the distribution of nutrient values observed in them will be without errors. Errors may be attributed to unmeasured stressors causing an incorrect reference designation, misclassified sites, unrecognized site classes in which natural conditions are unusual, or faulty measurements of the nutrient endpoints.

The percentile selected, and the criteria that may result, must conform to programmatic goals. The intended level of protection for designated uses should be clearly stated and used to justify the percentile selected and the proposed thresholds. The selection of a percentile of the reference condition as a criterion is defensible when it is supported by clear reasoning and corroborating analyses.

3.4 Stressor-response Analyses

Regression Interpolation

When a clear linear relationship is evident between a nutrient concentration and a response variable with an existing threshold, then a nutrient concentration can be associated with the response threshold through intersection with the linear regression. For example in a bi-plot of the macroinvertebrate NMMSCI in high elevation streams with smaller watersheds, the value of TN that corresponds to the NMMSCI threshold value (56.7 points) is easily determined as are ranges of TN values associated with error in the regression.

In this approach as in all others, variability that can be attributed to factors other than those being analyzed must be accounted for or otherwise recognized. To continue with the example above, relationships between nutrient concentrations and macroinvertebrate index values must include only sites for which the index is valid (high small streams), using consistent or comparable sampling methods, and factoring out any known sources of variability. Assessing sources of variability in the response variables that are not attributable to nutrients could include multiple regression with other stressor variables, partial correlation matrices, or regression of variables in reference sites to address residual classification variables.

Correlation Analysis and Bi-plots

The Spearman's rho correlation coefficient and bi-plots of nutrient concentrations and response metrics will be used to identify potential relationships between biological responses and nutrient variables. Correlation analyses identify the apparent linkages between biological condition and environmental variables. Bi-plots will be examined to determine if the correlations reflected a believable relationship.

Relationships of interest (those with high correlation coefficients) will be examined using a locally weighted regression line (LOWESS or loess) to describe the trend of metric change along the environmental gradients. LOWESS technique (Cleveland 1979) is designed to address nonlinear relationships where linear methods do not perform well. LOWESS combines much of the simplicity of linear least squares regression with the flexibility of nonlinear regression. It achieves this by fitting simple models to localized subsets of the data to build up a function that describes the deterministic part of the variation in the data, point by point. LOWESS fits segments of the data to the model, essentially, at the central tendency of the data. This method does not require specification of a global function of any form to fit a model to the data but to simply fit segments of the data to the model. We generally use a bandwidth that considered 75% of the data for smoothing the slope at each data point. The LOWESS regression line can be used

in combination with other indicators of nutrient thresholds of effect, primarily as a visual confirmation of changing biological measures at certain nutrient concentrations.

Change-point Analysis

The change-point is the point along an environmental gradient (nutrient concentrations) at which there is a high degree of change in the response variable (periphyton, chlorophyll a, or macroinvertebrate metrics). There are many ways to identify the change-point and each has advantages and limitations. We propose using two methods and determining the best application for each pair of stressor-response variables. The first method is the nonparametric deviance reduction (Qian et al. 2003, King and Richardson 2003), which works well when the response is stepped, or drastically changing at a recognizable point along nutrient concentration gradient. With this method, the data are divided into two groups, above and below a potential nutrient threshold, where each group is internally similar and the difference among groups is high. This technique is similar to regression tree models, which are used to generate predictive models of response variables for one or more predictors. Using this comparison, the change-point is the first split of a tree model with a single predictor variable (i.e., nutrient concentration). Output from this change-point analyses will include the threshold as well as confidence intervals estimated from a bootstrapping re-sampling technique. When both significant and nonsignificant change-points are identified, only significant change-points should be considered. Results can be tabulated and plotted for each site class, nutrient, and response variable and can be summarized as the median of significant change-points.

The second type of change-point analysis considers the slope of regressions on either side of a potential threshold (Qian and Cuffney 2012). This piecewise linear regression is appropriate to use when the data appear in a hockey stick pattern instead of a step function. The change point is identified as the point at which the two regression lines join, indicating a change in the response rate. Change-points can be calculated using R software (R Development Core Team 2010) and associated code.

One caveat of the change-point analysis is that a change-point may be identified, and even determined to be statistically significant, when the change-point value is actually only an artifact of the analysis and not an indication of a change in system properties (Qian and Cuffney 2012, Daily et al. 2012). The methods can find change-points, even in datasets with nearly straight line relationships between X and Y. It has been well established that nutrient concentrations limit algal growth as well as species composition. Therefore, it is reasonable to believe an ecological threshold does exist between certain periphyton metrics and nutrient concentrations. In our analyses, we will evaluate each relationship by examining the LOWESS fit on biplots of periphyton metrics and nutrient concentrations. If the LOWESS fit does not show a visually recognizable change at the identified change-point, then the change-point will be disregarded.

A second check on the general response pattern uses quantile regression to confirm limiting effects of nutrient concentrations on the response variable. Quantile regression is a method for estimating relationships between variables along the upper (or lower) boundary of a distribution of stressor-response data points (Cade et al. 1999). The quantile regression line represents biological potential (plotted on the y-axis) in relation to the stressor of interest (plotted on the x-axis). If limiting factors such as nutrients act as constraints on organisms, then the potential

maximum biological condition is observed as a sloping line on a wedge-shaped scatter plot of a biological metric against a nutrient variable. Points that are not along the slope of the wedge represent sites where biological condition is diminished by factors not represented on the x-axis, which in this case is nutrient concentration. We will use "R" software (R Development Core Team 2010) and associated code (quantreg) to estimate limiting relationships by quantile regression.

Several upper quantile regression lines (75th, 85th, 90th, and 95th) will be calculated and plotted. When the upper quantiles are relatively parallel, the biological potential is likely limited by the stressor variable (Cade et al. 1999). The multiple upper quantiles will be examined and indications of limiting effects will be determined based on the consistency of the slopes. When the 90th quantile regression line is consistent with the other lines, it can be plotted to illustrate the change in the potential biological resource for each increment of sediment disturbance. The quantile regression line may not be useful in identifying change-points because it shows only linear relationships.

Propensity Scores

We can use the propensity score approach to evaluate the plausibility that total phosphorus or total nitrogen causes true biological effects. The propensity score approach accounts for background effects of multiple co-varying stressors before indicating if there are effects of nutrient concentrations that are independent of the other stressors. This approach can be used to infer the cause of biological impairment and demonstrate that nutrient enrichment can impact the biological conditions (in general, not site-specifically).

The approach depends on identification of a number of streams with similar covariate distributions (other observed environmental factors), but which differ in nutrient concentrations. In the case of only a single factor (e.g., conductivity) covarying with nutrient concentrations, we could simply stratify the dataset by this factor, splitting the dataset into groups with similar values.

Propensity functions (Imai and Van Dyk 2004, Yuan 2010) summarize the contributions of all known covariates as a single parameter. A propensity function is defined as the conditional probability of a multivariate treatment (e.g., different nutrient concentrations), given values of known covariates. This conditional probability can be characterized by a single parameter, referred to here as the propensity score, which is the mean expected value of the treatment. For example, observed nutrient concentrations can be modeled as a function of covariate values using regression analysis, and the predicted mean nutrient concentration in each stream is the propensity score. Then, stratifying by propensity score effectively splits the dataset into groups with similar covariate distributions. Once the dataset is stratified, causal effects of nutrients can be more confidently estimated within each group because distributions of other covariates are similar (Yuan 2010). While effects thresholds can be identified, they would not be feasible to apply because of uncertainty in assigning new sites to propensity score classes.

The specific steps in a propensity score analysis include 1) identifying a suite of environmental variables that covary with nutrient concentrations, 2) using a generalized linear model (with

appropriately transformed values) to summarize the covariates and predict nutrient concentrations, 3) stratifying the predicted nutrient propensity scores into 2-5 different classes, corresponding to perceived changes in nutrient expectations along the propensity score axis, and 4) characterizing relationships between biological responses and nutrient concentration in each of the strata.

The environmental variables that should be included in the propensity score analysis are those that are related to TN and TP and to the response variable to be analyzed and that have continuous (not categorical) values. The variables could include conductivity, water temperature, alkalinity, chloride, sulphate, hardness, total suspended solids, turbidity, shading, flow, pH, DOC, and others. If total nitrogen is highly correlated with total phosphorus, effects observed on phosphorus after accounting for nitrogen are expected to be similar to effects of nitrogen after accounting for phosphorus. Therefore only effects with one nutrient species would be examined and similar effects on the other species would be implied. These analyses can be conducted with all data statewide because that strengthens the analysis and regional effects on nutrient concentrations would be accounted for through the covariants and sectioning of the propensity score gradient.

It is expected that the limitations on periphyton, chlorophyll a, or macroinvertebrates can become irrelevant when nutrient concentrations reach certain high levels. In other words, the response-nutrient relationship may be apparent when nutrients are limiting, but may not be apparent when nutrients are so plentiful that additional nutrients have no additional effect on the biota. The propensity score analysis may be best applied with periphyton or chlorophyll a data because the effects of nutrients on periphyton are direct.

Species Sensitivity Distributions

The Species Sensitivity Distribution (SSD) approach has been used to develop water quality criteria since the early eighties (Posthuma et al. 2002) using experimental data. Laboratory toxicity test detected responses (LC50) of a few species and these responses (sensitivities to toxicants) were then used to develop species sensitivity distributions. Water quality criteria derived using the SSD approach were based on dose-response relationships that examined the toxicity of the single constituent on a biological endpoint in a laboratory setting.

Recently, state and federal biomonitoring programs have accumulated ample species response data to allow testing of the SSD approach based on field observations (not just laboratory results). The field observed datasets have three notable advantages. 1) They are generally large dataset with multiple observations. 2) Hundreds of taxa were observed responding to various stressor gradients. 3) The criteria developed from this approach would be protective of individual taxa, not calculated metrics or indices.

The disadvantages of field observation are also evident. 1) Multiple stressors often exist concurrently and can confound response mechanisms. 2) Rare taxa (low capture probability, low abundance) may not be adequately incorporated into the analysis. 3) Systematic or random errors could be very large. While the SSD approach is a valuable way to develop nutrient thresholds and can provide an important line of evidence, we consider the use of the SSD approach to

derive nutrient criteria from field data as an experimental approach and have to be cautious when applying the results.

The SSD approach to developing numeric stressor criteria involves examination of the New Mexico data to find responses of each individual taxon to nutrient variables. Response curves of macroinvertebrate taxa along nutrient gradients can be described with Generalized Additive Models (GAM), which could be unimodal, decreasing, increasing, or U-shaped (concave-up). Both relative abundance and presence/absence of macroinvertebrate taxa can be used as responses. To decrease effects of co-occurring stressors, sites can be partitioned so that those with probable stress from non-nutrients can be excluded (e.g., sites with less than the 95th percentile of reference conductivity values).

After the relationships are determined, taxa tolerances to nutrients can be identified and modeled through analysis of the taxa distributions and nutrient concentrations. Finally, based on the tolerance of each taxon, the cumulative distribution function can be described for all observed taxa. This taxa tolerance distribution can be examined to determine the nutrient levels at which a significant numbers of taxa (e.g., 95%) are protected. The associated nutrient level would then be considered as a potential nutrient criterion. The SSD approach is exploratory and should be used for corroboration of other threshold development results, not as a sole or primary approach.

3.5 Synthesis of Criteria Ranges

The strength of an analysis with multiple approaches and response endpoints comes from the multiple lines of evidence. They can be used to show central tendencies and extremes in potential thresholds. The central tendency of potential thresholds shows corroborated evidence, which can give greater confidence in a selected threshold. However, the extreme values may be selected if the regulating agency has a reason to be more or less protective. This decision may be based on confidence in an individual analytical technique or on corroborating evidence from the scientific literature.

The ultimate decision on nutrient criteria from the range of possible thresholds lies with NMED. Their decision will need to be well communicated and justified transparently. To help with this, the potential thresholds from the proposed multiple analyses and the scientific literature should be tabulated for review. Each potential threshold should be listed with any caveats, error, and uncertainty associated with the method or the underlying data. In addition, interpretations of the protectiveness of certain thresholds should be described in terms of the designated uses.

4 Application

As outlined in the NMED nutrient criteria development plan (2006), nutrient threshold values can be used in assessing waters and protecting water quality through watershed management activities. Nutrient criteria can also be used to establish TMDLs and define source waste load allocations within NPDES permits and load allocations to address non-point sources of nutrient loading. When nutrient sources have been identified, resource managers can implement best management practices and other activities necessary to maintain or improve the designated uses of streams.

4.1 State Standards

The proposed analyses should be conducted with understanding of the requirements for adoption of criteria into state standards. Establishing nutrient thresholds according to this analysis plan will provide a scientific basis for defending the threshold values, which is necessary if they are to be incorporated into the *State of New Mexico Standards for Interstate and Intrastate Surface Waters* (20.6.4 NMAC). According to the Code of Federal Regulation (40 CFR 131.6), the minimum requirements for a water quality standards submission include 1) use designations, sufficient protective criteria and anti-degradation policies (or their revisions), 2) descriptions of methods used and analyses conducted, 3) certification by the state attorney general (or equivalent), and 4) information that will help the regulating agency determine adequacy of the scientific basis, as well as information on general policies. The submission process also includes opportunities for public comment and responses to those comments.

The chances for successful adoption of criteria will be improved if the analyses are shown to be scientifically defensible, transparent, reproducible, and protective. These elements will be enhanced if the following questions are addressed during analysis.

Defensibility

- Are the proposed criteria scientifically defensible?
- Has the theory, technique, hypothesis been tested?
- Can it withstand peer review?
- Is there a known error rate?
- Are there published standards for the techniques?
- Has the method/technique gained acceptance within the scientific community?
- Were accepted sampling protocols followed, laboratory methods, and data analysis methods used?
- Was a QA/QC process used and documented throughout?
- Are the designated uses of waters clearly articulated?
- Is there a clear discussion of how the criteria protect the designated uses?

Transparency

- Is the criteria process clearly laid out?
- Are the steps of the analysis clearly stated?

- Is documentation of the process readily available?
- If data or analyses were withheld, was this justified?
- Reproducibility
 - Could the analysis be replicated?
 - Were the methods clearly documented and the samples archived?
 - Are the data readily available for analysis?
 - Are the analyses readily available for review?
- Protectiveness
 - Are the resultant criteria or proposed values likely to protect the designated uses of waterbodies or are they unlikely to be appropriately protective?
 - Is the level of protection clearly defined?
 - Is this determination supported by professional opinion and by logical interpretation of the analyses?

4.2 Application in Assessment Programs

We do not propose any application procedures other than those already outlined in the NMED Procedures for Assessing Water Quality Standards Attainment (2011). For clarity as we develop potential criteria, the existing procedures are re-stated here.

An assessment unit is **Fully Supporting** with respect to New Mexico's narrative nutrient standard if (1) one or none of the variables exceed their threshold value, or (2) both total nitrogen and total phosphorus exceed their threshold values, but there was no indication of a biological response to elevated nutrients (i.e., the response variables did not exceed their threshold values). In this instance a review of the ecoregional thresholds may be warranted. An AU is **Not Supporting** if at least one causal variable and one response variable exceed their thresholds.

The causal variables include total nitrogen and total phosphorus, with applicable thresholds in Table 1. The response variables include dissolved oxygen, pH, and chlorophyll *a*, with applicable thresholds in Section 1.5.

5 Proof of Concept

5.1 Data Reduction

All of the data should be considered for analysis, but some analyses are better suited to specific data types or summaries. For example, in the NMED dataset, there are multiple samples collected at the same site over time. The geometric mean or maximum of nutrient values per site can be used to describe value distributions and for site classification. The geometric mean reduces the effect of high outlier values when compared to a simple mean. Maximum values capture worst-case scenarios.

For stressor-response relationships, the chemistry and response samples should be limited to those within one month of each other. If multiple chemistry samples were collected during that period, the sample collected closest to the response sample should be used. Only a single stressor-response dataset should be used per site.

As described above (Section 3.4), the response data can be analyzed as metrics of the assemblage or series (for diel dissolved oxygen data). The biological metrics are usually limited to those that are basic and familiar summary metrics or are known indicators of stress. Since there are more ways to measure an assemblage than there are meaningful ways to interpret several metrics, it is recommended that the number of metrics analyzed should be limited through a preliminary screening process that discerns familiar, proven, precise, or sensitive metrics. Metrics that have high measurement error or are unresponsive to stress are lower priority for stressor-response analyses.

NMED

The NMED nutrient data were collected mostly in non-winter months (Figure 2). Because there may be seasonal effects on nutrients due to discharge or fertilization patterns, light intensity, and plant uptake, the samples collected in the winter (Dec, Jan, and Feb) should be removed from the general analysis. In the NMED dataset, nutrients show some bias in the winter months (N is high and P is low) (Figure 3). Seasonal effects on nutrient concentrations should be considered and analyzed in future analyses. The NRSA and WSA + EMAP samples were collected within a narrower window of time, minimizing seasonal effects.

Comment [pc8]: Interesting observation. Do you think analyses should be done with and without these months? Also, how would sites be assessed during these months if forthcoming criteria or translators did not include winter months?

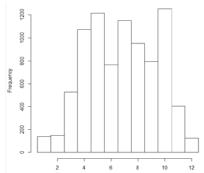


Figure 2. Sampling frequency by month (NMED data).

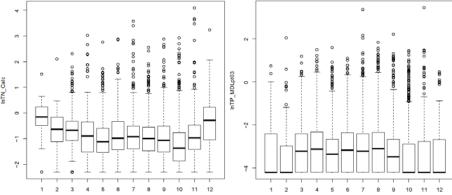


Figure 3. TN (left) and TP (right) concentrations by month (NMED data).

5.2 Reference Sites

GIS analysis

After QA/QC of the site list provided by Tetra Tech to EPA Region &6, the following GIS analysis was conducted. EPA Region &6 staff (Robert Kirkland and Angel Kosfisher) snapped 393 sites to NHDPlus 1.0 flowlines for further analysis. All required data for the landscape computations were downloaded, including National Hydrography Dataset (NHD) Plus data for the following NHD Plus Production units; 11b,11d,11e, 12b, 12f, 13a,13b,14a,15a,15b. For each site, a 1.0 mile buffer around the sampled reach was delineated. A more refined analysis would include delineation of the catchment area of the site, which would allow analysis of landscape characteristics in the contributing watershed. The buffer was used in this preliminary analysis in

the interest of time. However, the reach on which the site is located includes downstream sections, and the characteristics in and around the downstream sections may not actual influence conditions at the sampled site. Therefore, conditions in the buffer of a site could indicate reference quality if stressors were absent, but existence of stressors did not necessarily negate potential reference status.

Landscape characteristics were determined for point values with spatial join (vector) or extract values to points (raster). The 1 mile buffers were used for calculation of areal extent of surrounding landscape characteristics. Analyzed variables were related to climate, soils, geology, elevation, slope, stream order (Strahler 1957), land use, and roads (Table 4).

Table 4. Variables used in GIS analysis.

| Variable | Description | | |
|---------------------------------|---|--|--|
| Point Values | | | |
| Stream Slope | NHD Plus join with flowline attributes table | | |
| Stream Order | NHD Plus join with NHDFlowlineVAA table | | |
| Elevation (cm) | NHD Plus DEM files | | |
| Land Slope | ARCGIS Spatial Analysis Slope tool | | |
| Permeability | STATSGO Permeability join with soil layer table | | |
| Designated Use | RAD 305b Assessed Segments joined with ATTAINS data | | |
| Precipitation | PRISM | | |
| Temperature | PRISM | | |
| Canopy | NLCD 2001 Canopy Cover | | |
| Level 3 and 4 Ecoregions | EPA Ecoregions | | |
| Geology | USGS Integrated Geological Map | | |
| Watershed Values | | | |
| Road density | Attila tool and TIGER 2000 files | | |
| Number of road/stream crossings | ARCGIS tools | | |
| Land Use and Cover | Attila tool and NLCD 2006 data | | |
| Canopy Density | Attila tool and NLCD 2001 Canopy data | | |

Sites with 100% natural land uses in a 1.6 km buffer around the reach of the site were given a designation of Candidate Reference ("RefCand") if they were not otherwise identified as reference. This added 82 potential reference sites to the 102 that were identified through past projects. These sites should be reviewed by NMED and the reference status confirmed. Sites were not taken off the reference list because with the simplified GIS analysis, the active land uses may be downstream, having little effect on the site.

5.3 Classification

The GIS data derived for this preliminary analysis is not specific enough for a detailed classification analysis. The buffer around the sampled reach and the reach itself may be small or downstream of the contributing watershed of the site. For example, slope was measured in the reach. If the reach was short, then the beginning and end of the reach may appear to have the

same elevation, due to coarse resolution of contours. The reach would then appear to have a slope of 0%, although the actual slope could be greater.

Site classes for the proof of concept were assumed to be as described in the NMED Procedures for Assessing Water Quality Standards (2011), defined by ecoregions and designated uses. Refined site classes for nutrient assessment may continue to use the existing classes because of the need to address designated uses in standards. Otherwise refinements would proceed as demonstrated in the New Mexico sediment threshold development (Jessup et al. 2010), with PCA and correlations of nutrient and environmental variables.

5.4 Nutrient Distributions

Nutrient distributions in reference and all NMED sites are shown for TN and TP in categories of nutrient regions and designated uses (Figure 4 through Figure 9). In most cases, the distribution of TN concentrations in reference sites overlaps substantially with the distribution of all sites. Distributions of TP concentrations are somewhat lower than in all sites. It is evident that with the current site categories and reference designations, using the 75th percentile of reference values would give very different thresholds than the 25th percentile of all sites.

To ensure that distribution statistics could be used to derive valid potential thresholds, the points going into the distribution should be examined for sources of variability. This would include reference site determination, site classification, season, flow, canopy, and other variables. The number of reference sites from which statistics could be derived should be adequate to represent the central tendencies and ranges of values. With fewer than 10 points, the percentile values from distributions are subject to data bias. Larger datasets will give more stable, repeatable percentile values.

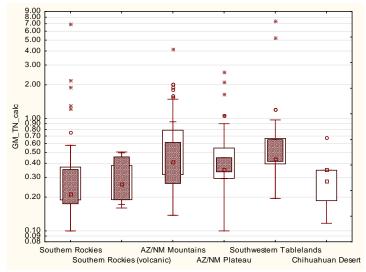


Figure 4. Total nitrogen distributions in all NMED sites (open interquartile boxes) and reference sites (interquartile boxes with hachures) for nutrient regions and all designated uses.

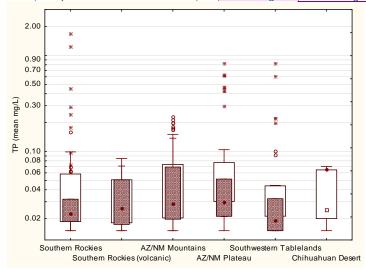


Figure 5. Total phosphorus distributions in all NMED sites (open interquartile boxes) and reference sites (interquartile boxes with hachures) for nutrient regions and all designated uses.

Comment [pc9]: Do you mean level III ecoregions? Need to define points and ranges on this amd subsequent figures.

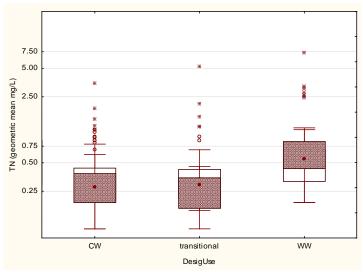


Figure 6. Total nitrogen distributions in all NMED sites (open interquartile boxes) and reference sites (interquartile boxes with hachures) for designated use categories in all nutrient regions.

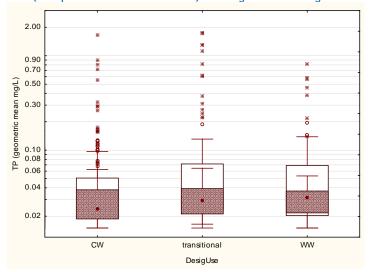


Figure 7. Total phosphorus distributions in all NMED sites (open interquartile boxes) and reference sites (interquartile boxes with hachures) for designated use categories in all nutrient regions.

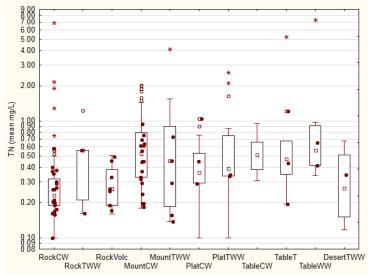


Figure 8. Total nitrogen distributions in all NMED sites (box plots) and reference sites (solid points) for designated use and nutrient region categories.

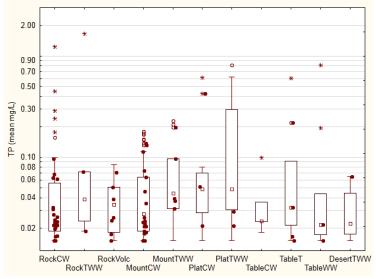


Figure 9. Total phosphorus distributions in all NMED sites (box plots) and reference sites (solid points) for designated use and nutrient region categories.

5.5 Linear regression

For this analytical approach, the nutrient concentration and response variable pairs from which nutrient thresholds could be derived include TN and TP with all response variables that have existing thresholds, such as chlorophyll a and the benthic index in the mountains. In an example using NRSA data, we assumed that the Western Mountains as defined by NRSA were similar to the Southern Rockies site class (Figure 10). With an existing chlorophyll a threshold of 3.9 mg/L (Table 2), the corresponding TN threshold would be 0.49 mg/L, derived by intersecting the chlorophyll a threshold with the regression line. A second example with TP shows that the existing chlorophyll a threshold of 8.2 mg/L (Table 2) corresponds to a TP threshold of 1.2 mg/L (Figure 11).

When we attempted to regress chlorophyll *a* on TN and TP in the NMED data (Figure 12 and Figure 13), the relationships were not as distinct as is shown in the NRSA data. This may be due to high variability among site types or secondary factors that need further dissection. Site class, shading and current velocity may be such factors.

The relationship between nutrient concentrations and the benthic macroinvertebrate index in cold water high elevation small mountain sites shows that regression on the NMMSCI may be possible (Figure 14 and Figure 15). However, in this preliminary analysis, the upper confidence limit of the regression equation does not intersect with the high small NMMSCI threshold. This suggests that the relationship is not well defined in sites with higher nutrient concentrations. The TN and TP concentrations associated with the benthic index threshold are 0.55 and 0.22 mg/L, respectively.

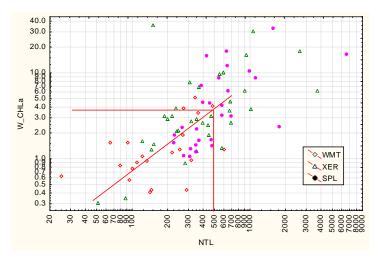


Figure 10. Regression of water column chlorophyll *a* on total nitrogen in NRSA data, showing the NRSA site types. Lines represent the regression equation in the Western Mountains (WMT) sites (diagonal), the intersection with the Southern Rockies chlorophyll *a* threshold (horizontal), and the corresponding TN threshold (vertical).

Comment [pc10]: You probably mean ug/l. Are you referring to water column chl a? Or benthic chl a, which would be in different units?

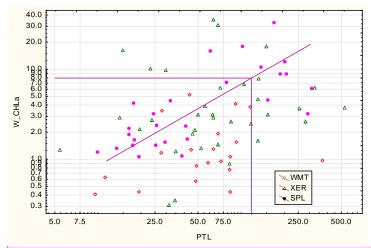


Figure 11. Regression of water column chlorophyll *a* on total phosphorus in NRSA data, showing the NRSA site types. Lines represent the regression equation in the Southern Plains (SPL) sites (diagonal), the intersection with the SW Tablelands chlorophyll a threshold (horizontal), and the corresponding TN threshold (vertical).

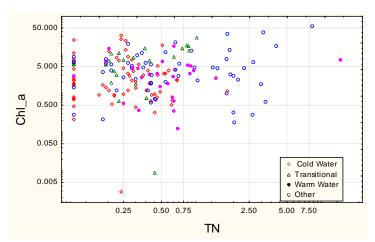


Figure 12. Chlorophyll *a* and total nitrogen in NMED data, showing the designated uses.

Comment [pc11]: I'm lost and think other readers will be put off by technical jargon rather than clear explanation.

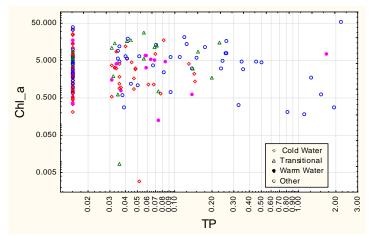


Figure 13. Chlorophyll a and total phosphorus in NMED data, showing the designated uses.

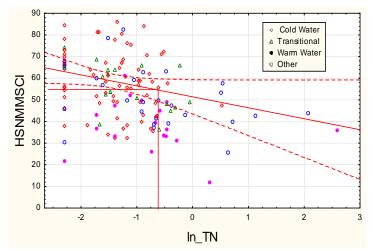


Figure 14. Regression of the benthic index (high small NMMSCI) on total nitrogen (natural log transformed) in NMED data, showing designated uses. Lines represent the regression equation in the cold water sites (diagonal, with confidence limits), the intersection with the Southern Rockies chlorophyll *a* threshold (horizontal), and the corresponding TN threshold (vertical).

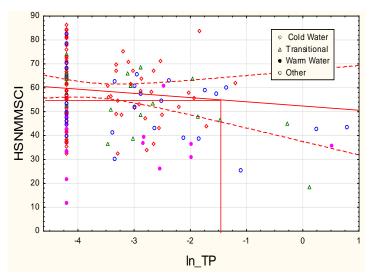


Figure 15. Regression of the benthic index (high small NMMSCI) on total phosphorus in (natural log transformed) NMED data, showing designated uses. Lines represent the regression equation in the cold water sites (diagonal, with confidence limits), the intersection with the Southern Rockies chlorophyll *a* threshold (horizontal), and the corresponding TP threshold (vertical).

5.6 Correlations

Correlation analysis was conducted within site classes and datasets. The example is from the NRSA data in the three site classes defined by the EPA analysis (Table 5). This correlation includes sites with all stressor conditions and is therefore most appropriate to consider in the context of the entire nutrient gradient. Another correlation analysis can be conducted within reference sites alone to discern natural gradients for classification or to determine residual variability after classification.

The correlations with TN in the western mountains show that there is a relationship between nitrogen and suspended materials (TSS and turbidity) as well as dissolved organic carbon (DOC). As expected, TN is also related to the stressor conditions in the riparian zone (W1_HALL) and the chlorophyll a in the water column (W_CHLa). TN and TP were not significantly related. Relationships with TP were not as expected in some cases, as shown by negative correlation coefficients with conductivity, salts (CA, MG, SO4), and Fish Cvr-Filamentous Algae (XFC_ALG). These relationships may be driven by outliers, may be due to a relatively short nutrient gradient in the mountains, or may be due to spurious bias in the dataset. These relationships need further investigation.

Relationships between nutrients and environmental variables were as expected and somewhat similar in the xeric and plains regions. TN and TP were correlated at significant levels in xeric areas and the plains. The relationship between nutrients was not strong in the mountains or xeric

Comment [pc12]: ??

areas, which is somewhat surprising and needs further analysis. In relation to natural variables, it appears that nutrients are lower in the east and higher in larger streams with slower current velocities. In the plains, nutrients were strongly related with salts (conductivity, choride, and sodium) and riparian conditions. The effects of flow were not significant in the plains, although indicators of stream size were.

These relationships can lead to insights regarding sources of nutrients, covariants, and multiple stressor effects. For example, propensity scores (Section 5.8) would be calculated based on covariants of nutrient concentrations. Further analysis can continue using partial correlation, refined site classification, and propensity score analysis. With partial correlation, variables that covary with nutrient concentrations can be factored out to discover relationships between nutrients and responses that are due primarily to nutrients. For example, TN and TP are correlated to TSS in almost all regions and nitrogen is related to dissolved organic carbon (DOC) in all regions. By accounting for these variables first and then looking for remaining effects of nutrient concentrations on periphyton (for example), then the effects of nutrients alone can be detected. It may be that the variables are so strongly correlated that remaining relationships are not detected.

Table 5. Pearson correlation coefficients for TN and TP (log transformed) and environmental factors by EPA site class (NRSA data). WMT = Western Mountains, XER = Xeric, and SPL = Southern Plains. Values shown in red font were significant (p<0.05).

| Variable | Variable | WMT, N = 23 | | XER, N = 35 | | SPL, N = 29 | |
|-----------|--------------|-------------|-------|-------------|-------|-------------|-------|
| category | | TN | TP | TN | TP | TN | TP |
| Chemistry | InNTL | | 0.30 | | 0.38 | | 0.78 |
| Chemistry | InPTL | 0.30 | | 0.38 | | 0.78 | |
| Physical | XLAT_DD | -0.08 | 0.13 | -0.33 | -0.02 | -0.17 | -0.08 |
| Physical | XLON_DD | -0.31 | 0.31 | -0.41 | -0.05 | -0.58 | -0.47 |
| Physical | WSAREA_KM2 | -0.04 | 0.12 | 0.37 | 0.44 | 0.43 | 0.19 |
| Response | P_CHLa | 0.36 | -0.20 | 0.40 | -0.12 | 0.59 | 0.29 |
| Response | W_CHLa | 0.51 | 0.12 | 0.30 | 0.03 | 0.59 | 0.58 |
| Physical | Flow_CFS_All | 0.21 | 0.03 | -0.06 | 0.12 | -0.28 | -0.27 |
| Chemistry | DO | 0.39 | -0.13 | -0.18 | -0.06 | -0.07 | -0.15 |
| Chemistry | pH field | 0.09 | 0.10 | -0.22 | -0.21 | 0.00 | 0.08 |
| Chemistry | pH lab | 0.05 | -0.26 | -0.34 | -0.17 | -0.53 | -0.46 |
| Physical | TEMPERATURE | 0.15 | -0.21 | 0.06 | 0.03 | 0.07 | 0.11 |
| Chemistry | COND | -0.15 | -0.57 | 0.44 | -0.18 | 0.83 | 0.69 |
| Chemistry | AL | 0.10 | 0.21 | 0.23 | 0.38 | -0.15 | -0.04 |
| Chemistry | ANC | -0.12 | -0.35 | 0.13 | -0.02 | 0.37 | 0.30 |
| Chemistry | CA | -0.07 | -0.58 | 0.34 | -0.34 | 0.32 | 0.10 |
| Chemistry | CL | -0.36 | -0.37 | 0.44 | -0.11 | 0.79 | 0.73 |
| Chemistry | COLOR | -0.04 | 0.11 | 0.06 | 0.40 | 0.10 | 0.25 |
| Chemistry | DOC | 0.49 | 0.28 | 0.46 | 0.27 | 0.50 | 0.45 |
| Chemistry | MG | -0.03 | -0.56 | 0.33 | -0.26 | 0.54 | 0.28 |
| Chemistry | SODIUM | -0.30 | -0.32 | 0.44 | -0.16 | 0.86 | 0.76 |
| Chemistry | NH4 | 0.32 | -0.25 | 0.50 | -0.05 | 0.32 | 0.27 |

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Comment [pc13]: ??

| Chemistry | NO2 | 0.30 | -0.10 | 0.44 | -0.05 | -0.11 | -0.23 |
|-----------|----------------|-------|-------|-------|-------|-------|-------|
| Chemistry | NO3 | 0.48 | 0.00 | 0.69 | 0.15 | 0.69 | 0.38 |
| Chemistry | SIO2 | 0.00 | 0.36 | -0.03 | 0.13 | 0.08 | -0.02 |
| Chemistry | SO4 | 0.01 | -0.67 | 0.36 | -0.28 | 0.39 | 0.16 |
| Physical | TSS | 0.67 | 0.36 | 0.38 | 0.65 | 0.52 | 0.65 |
| Physical | TURB | 0.63 | 0.23 | 0.34 | 0.60 | 0.26 | 0.41 |
| Physical | XBKF_W | -0.09 | 0.07 | 0.09 | 0.48 | 0.46 | 0.62 |
| Physical | XWIDTH | -0.03 | 0.13 | 0.16 | 0.51 | -0.10 | 80.0 |
| Physical | XBKF_H | -0.13 | 0.13 | 0.16 | -0.05 | -0.20 | -0.08 |
| Physical | BFWD_RATIO | -0.14 | -0.02 | 0.02 | 0.37 | 0.43 | 0.58 |
| Response | XFC_ALG | -0.14 | -0.75 | -0.23 | -0.15 | -0.18 | -0.31 |
| Physical | PCT_FN | 0.38 | 0.24 | 0.26 | 0.01 | 0.06 | 0.10 |
| Physical | PCT_SAFN | 0.38 | 0.30 | 0.29 | 0.25 | 0.35 | 0.22 |
| Physical | LRBS_G08 | -0.26 | -0.37 | -0.06 | -0.12 | -0.06 | 0.06 |
| Physical | XSLOPE | -0.08 | -0.51 | -0.40 | -0.42 | -0.25 | -0.34 |
| Condition | W1_HALL | 0.56 | -0.01 | 0.11 | 0.01 | -0.38 | -0.43 |
| Condition | W1_HAG | 0.39 | -0.06 | 0.01 | -0.05 | -0.41 | -0.42 |
| Physical | V4W_MSQ (wood) | -0.59 | -0.29 | -0.22 | 0.01 | | |
| Physical | XCanopy | -0.05 | 0.03 | -0.18 | 0.19 | 0.03 | 0.05 |
| Physical | PCT_FAST | 0.01 | 0.01 | -0.36 | -0.28 | 0.06 | 0.10 |
| Physical | PCT_SLOW | -0.01 | -0.01 | 0.36 | 0.28 | -0.06 | -0.09 |
| Physical | PCT_POOL | 0.02 | -0.19 | -0.08 | -0.05 | -0.19 | -0.11 |

5.7 Change-point Analysis

For the proof-of-concept, change-point analysis was attempted in two datasets (NRSA and NMED), three response assemblages (chlorophyll *a*, periphyton, and benthic macroinvertebrates), and two techniques (deviance reduction and segmented regression). All attempts did not give clear indications of change-points. For these preliminary illustrations, those relationships that show promise are included. Additional analyses would be necessary for the complete derivation of nutrient criteria, including LOWESS regression, quantile regression, and sub-setting or adjusting variables based on site classification.

Change-point analysis with deviance reduction results in plausible, significant and relatively precise change-points for TN and TP derived from chlorophyll *a* in the NRSA dataset (all sites combined) (Figure 16). Change-point analysis with segmented regression does not confirm the change-point identified through deviance reduction (Figure 17). Because the two segments of the segmented regressions have similar slopes, the segmented regressions suggest that there are no distinct change-points and that changes in chlorophyll *a* occur all along the nutrient gradients. LOWESS regression lines might give additional interpretive information, while quantile regression would probably confirm the continuous gradient suggested by segmented regression.

Several periphyton metrics were compared to nutrient concentrations in the NRSA data (all sites combined). Change-point analysis with deviance reduction generally resulted in change-points with wide confidence intervals (Figure 18). Across all metrics, the range of change-point estimates was $23-193~\mu g/L$ for TP and $148-919~\mu g/L$ for TN. In addition, the segmented regression change-points generally did not confirm the deviance reduction change-points.

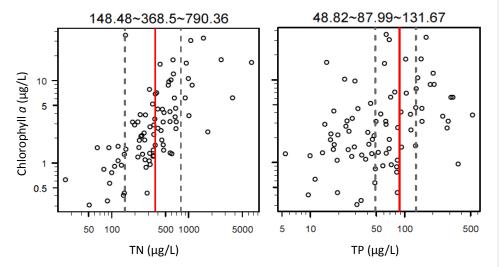


Figure 16. Deviance reduction change-point analysis for nutrient concentrations and the water column chlorophyll *a* (NRSA data). This shows change-point values of 368 and 88 μg/L for TN and TP, respectively, and the 95% confidence intervals for the change-point estimates.

Comment [pc14]: Is this data for entire state? Should the analyses be conducted by region rather than state-wide? Ditto for subsequent figures.

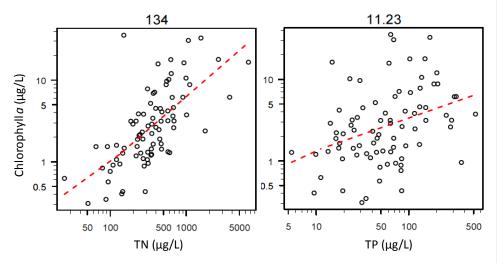


Figure 17. Segmented regression change-point analysis for nutrient concentrations and the water column chlorophyll a (NRSA data). This shows change-point values of 134 and 11 μ g/L for TN and TP, respectively.

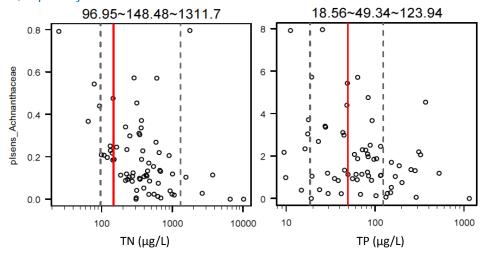


Figure 18. Deviance reduction change-points for nutrient concentrations and one periphyton metric (percent individuals of sensitive taxa in the family Achnanthaceae, NRSA data). This shows change-point values of 148 and 49 μ g/L for TN and TP, respectively, and the 95% confidence intervals for the change-point estimates.

In the NMED dataset, nutrient concentrations were compared to the NMMSCI and 15 other benthic macroinvertebrate metrics. In addition to the NMMSCI (Figure 19), 12 of the 15 metrics tested resulted in segmented regression change-points of 0.75~mg/L TN (ranging down to 0.46~mg/L). For TP, the NMMSCI and 4 of the 15 metrics indicated a change-point at 0.18~mg/L. The range of change-point values was broad across metrics: 0.06-0.3~mg/L TP. This example shows that the range of values for the NMED nutrient-macroinvertebrate dataset mostly includes low nutrient concentrations, several with concentrations below the standardized detection limits.

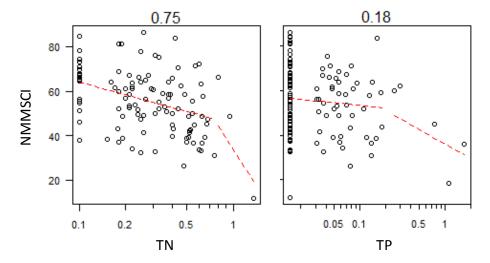


Figure 19. Segmented regressions for nutrient concentrations and the benthic macroinvertebrate index (NMMSCI), showing the change-point values of 0.75 and 0.18 mg/L for TN and TP, respectively.

5.8 Propensity Scores

The proof of concept for the propensity score analysis is from the Montana statewide nutrient analysis (Tetra Tech 2010). In the example, six variables were used in a generalized linear model on log transformed data (except temperature) to predict TP. Predicted TP (propensity scores) were stratified into four different classes, corresponding to perceived changes in TP expectations along the propensity score axis (Figure 16). Correlations of the variables in the model with the propensity scores (Table 6) indicate that Class 1 (left of the first vertical line in Figure 16) has the lowest TSS and TKN, as well as lower conductivity, alkalinity, temperature, chloride, and hardness and somewhat higher chlorophyll *a* and dissolved oxygen. Sites with greater degrees of stress are in Class 4 (furthest right in the figure). Correlations of TP with covariates were greatly reduced within the classes in comparison to correlations in all sites (pooled classes). Correlations of TP with TN and TKN remained relatively high in the individual classes.

Periphyton responses to TP were examined using a responsive multimetric index, the WEMAP MMI. The MMI increases with increasing nutrient stress. The relationship was characterized through TP-MMI bi-plots within propensity score classes, with the LOWESS regression line and change-point analysis superimposed on the graph. As expected, periphyton responses to TP were evident in the classes with lower stressor intensities, where a change-point with relatively narrow confidence intervals could be identified in association with a change in the LOWESS curve (Figure 17).

In Class 4, where virtually all of the TP values were greater than 0.03 mg/L, there was no obvious trend or change-point in periphyton MMI values along the TP gradient. These results suggest that TP had an effect on periphyton when TP and background stressors were less than 0.03 mg/L. At higher values, additional TP did not change the periphyton characteristics, perhaps because nutrients were no longer limiting at high levels. The change-points identified in Classes 1 – 3 suggested that effect thresholds may occur when TP was between 0.013 and 0.028 mg/L, depending on background expectations that may be accountable through site classification. When ecoregions were associated with propensity scores, there was no discernable pattern to suggest that propensity score classes and ecoregions were aligned. If they had been aligned, then ecoregions would be accounting for the same factors considered in developing the propensity scores. However, they accounted for different factors, and this did not imply that one was more accurate than the other.

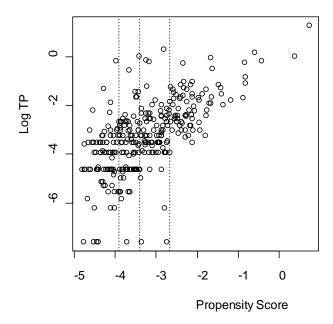


Figure 20. Scatterplot of propensity scores versus log TP. The samples were stratified into four classes, delineated by the vertical dashed lines.

Table 6. Correlations (Spearman rho) of Total Phosphorus with environmental covariates in all groups and in four propensity classes.

| | All | | | | |
|------------------|---------|---------|---------|---------|---------|
| | samples | Class 1 | Class 2 | Class 3 | Class 4 |
| Conductivity | 0.306 | 0.087 | -0.151 | 0.011 | -0.105 |
| Temperature | 0.360 | 0.197 | -0.261 | 0.093 | 0.048 |
| Alkalinity | 0.348 | 0.090 | -0.207 | 0.051 | -0.083 |
| Chloride | 0.274 | 0.229 | -0.078 | -0.069 | -0.147 |
| Hardness | 0.256 | 0.087 | -0.282 | 0.007 | -0.167 |
| TSS | 0.611 | -0.046 | 0.205 | -0.054 | 0.436 |
| TKN | 0.653 | 0.424 | 0.316 | 0.356 | 0.597 |
| Chlorophyll a | -0.121 | -0.229 | 0.110 | -0.026 | -0.081 |
| Dissolved oxygen | -0.162 | -0.078 | -0.066 | -0.113 | 0.004 |
| pН | 0.168 | 0.098 | -0.159 | -0.241 | -0.001 |
| TN | 0.617 | 0.402 | 0.317 | 0.411 | 0.560 |

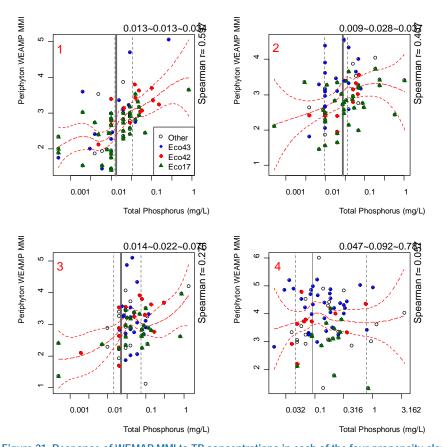


Figure 21. Response of WEMAP MMI to TP concentrations in each of the four propensity classes. The vertical lines are the change points and their 90% confidence limits.

5.9 Species Sensitivity Distributions

Because it is extremely intensive to perform, the proof of concept for species sensitivity distributions relies on an existing analysis from Montana (Tetra Tech 2010). The most robust approach for identifying tolerance values of individual taxa to nutrient concentrations was the 95% cumulative probability of the response curve from general additive models for each taxon (Model95). The probability of occurrence of *Acentrella* responded to both TN and TP gradients (Figure 18). *Acentrella* presents almost linear decreasing response to elevated TP concentrations and a unimodal response to TN concentrations. Two other methods were also attempted for developing tolerance values (95th cumulative percentile tolerance from abundance [CP95], 95th percentile of maximum observed stressor value [95thMax]), but they were less robust.

After the taxa tolerance values were developed, a selected list of taxa was used to generate an empirical distribution function (Figure 19). The nutrient concentrations associated with the 5th percentiles of cumulative frequencies were considered potential criteria to protect 95% of the taxa.

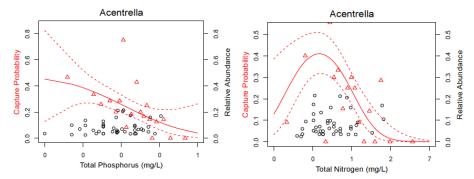


Figure 22. Examples of *Acentrella* response to nutrient gradients. The black circles represent the relative abundance of *Acentrella* in a site. The red triangles represent capture probabilities of the taxon. The red dotted lines are the mean model fit and its 90% confidence intervals of the capture probability.

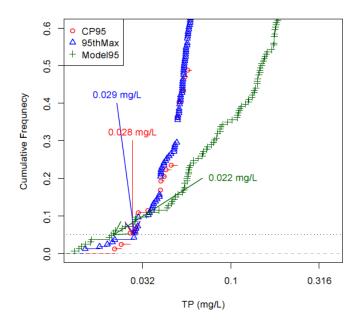


Figure 23. Cumulative frequency distribution of macroinvertebrate sensitivity to the TP gradient when other stressors were excluded. The 95th cumulative percentile tolerance from abundance (CP95), 95th percentile of maximum observed stressor value (95thMax), and presence/absence based generalized additive model derived tolerance values (Model95) were used to derive the cumulative frequency curves.

6 Discussion

6.1 Datasets

- There is a need to associate site characterization, designated uses, and reference criteria
 to all sites in the GIS analysis and the NMED dataset. This would allow refinement of the
 reference site identification and site classification analyses to include more sites in
 appropriate contexts.
- Data comparability analysis should be conducted to determine opportunities for pooling data across datasets. This may only pertain to the nutrient data and not the response data because of disparate data elements and collection techniques.
- Some datasets were not fully developed, such as diel dissolved oxygen records for NMED. Also, periphyton and macroinvertebrate metrics need further explanation in the NRSA dataset. Additional periphyton metrics may be warranted for NRSA data and periphyton metric calculation is needed for NMED data.
- The NMED data were compiled from multiple sources (different programs over time).
 While NMED suggests that all of these sites and time periods are valid for pooling, this might need investigation for some variables or certain analyses.
- A complete data dictionary should be prepared and associated with the compiled dataset.

6.2 Literature review

- Review literature for regional studies of conditions and stressor-response relationships
 - Literature should be regionally specific when available
 - Literature should be provided with the report for additional review
 - Derivation of literature values must be thoroughly explained
 - Linkage of literature values to protection goals must be clear
 - Literature review is incomplete in this analysis plan and proof-of-concept
- Analyses should be placed in a literature context and analytical results should be compared to expectations and precedents

6.3 Nutrient Concentration Distributions

- Reference site criteria must be consistently applied, based on variables that are independent of nutrient concentrations
 - Selection of reference sites must be consistent and defensible
 - Selection of thresholds for variables must be clear
- Reference site caveats
 - If there are stark differences in nutrient concentrations in reference sites across site classes, are the reference criteria appropriate or equitable?

- Is land use a confounding factor?
 - Why should nutrients/criteria vary across classes?
 - Are effects really so different over the spatial scales?
 - Is land use intensity correlated with natural landscape characteristics?
- More refined GIS analyses are needed to establish high quality reference sites
 - Is there a complete stressor gradient over which to detect responses?
 - Some analyses are sensitive to the range of nutrient values

• Site classification

- Consider natural conditions that effect nutrients but are not accounted for through categorical classes
- The existing nutrient classes or similar ones that incorporate landscape characteristics and designated uses should be further developed
- More refined GIS analyses may be needed to continue with classification

Analytical projections

- Sub-sets of the datasets may be appropriate for analysis due to reduced variability in sampling bias or resolution. Such variability may be associated with data sources, sampling programs, dates, etc.
- NRSA and WSA-EMAP data may be pooled in some analyses
- Reference site designation and site classification should be considered simultaneously or iteratively so that sufficient sample sizes are identified from which to derive thresholds from percentiles.

6.4 Stressor response

Most frequently used methods may be most robust

- Linear or non-linear regressions
 - Regression interpolation for known impairment conditions
- Change-point Analysis
 - Deviance reduction or piecewise linear regression
 - In our proof-of-concept, the two analyses were not always in agreement
- Ouantile and LOWESS regression for confirmation of change-points
- Visual interpretation of bi-plots and distributions
- Propensity scores and species distribution analysis are experimental methods

Always include ranges of potential effects and measures of uncertainty

- Uncertainty can be associated with repeated measurements, relationships, and analyses
- Statistical associations may not be biologically relevant and do not prove cause and effect
- The data must be adequate for the analyses (sufficient sample size, expected range of conditions, complete information)
- In the proof-of-concept, it appears that some datasets have limited ranges of nutrient concentrations, for which a change point may be difficult to identify

Account for site classes when possible and plausible

- The importance of site classes to effects must be established
- Analysis may be weak if it is too finely segmented or too coarsely grouped
- Consider residual variation after classification (partial correlation)

Unexplained variation can result in inaccurate numeric criteria

• Be clear on protection goals

- Use existing criteria of thresholds (when they exist) for associations
- Are we protecting at the level of impairment, or before impairment is evident?

• Select response variables purposefully

- Selecting response variables that relate directly to measures of designated use are
 most appropriate since criteria must ensure protection of the designated uses. The
 coupling of response variables to designated uses must be clear and the rationale
 explained.
- Plants (algae and macrophytes) respond directly to nutrient enrichment
- Macroinvertebrates and fish respond to secondary effects of nutrient enrichment, but may nevertheless show clear responses to nutrients

6.5 Multiple lines of evidence

• All analyses should be presented

- The results that suggest a narrow range of criterion values can be emphasized
- Any nonconforming results need also to be discussed
- Few lines of evidence could be sufficient (2 4 including literature support)
- Reference distribution evidence may be secondary to stressor-response evidence
- Combining two or more poor pieces of information to try and make one good piece is not acceptable
- When properly determined, statistical associations can be very useful in supporting a
 cause and effect argument as part of a weight of evidence approach to criteria
 development.
- The final document should provide greater detail on the implementation of statistical
 procedures and development of other supporting information to minimize the degree of
 unexplained variation and maximize the potential for the empirical stressor-response
 approach to result in useful numeric nutrient criteria.

6.6 Scientific Justification

Completeness

Do not withhold data or analyses

Reproducibility

- Provide adequate detail for repeating any analyses
- Clearly document methods and archive samples
- Provide data and analyses in formats readily available for analysis and review

Protectiveness

In professional opinions of the workgroup, are the resultant criteria or values proposed likely to protect the designated use of waterbodies described in the criterion proposal or are they unlikely to be appropriately protective?

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